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Chemical Constituents of *Euphorbia sanctae-catharinae* Fayed Essential Oil: a Comparative Study of Hydro-distillation and Microwave-Assisted Extraction

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ABSTRACT

Objective: Microwave-assisted extraction (MAE), as an effective tool for the extraction of essential oil from medicinal and aromatic plants, has gained a great attention over the last years, thus in this study, MAE was performed to extract essential oils from aerial part of *Euphorbia sanctae-catharinae*. **Methods:** microwave-assisted extraction (MAE), as well as hydro-distillation (HD) techniques was used for oil preparation. Oil composition was studied by gas chromatography-mass spectrometry analysis. **Results:** Essential oil constituents and percentages of the obtained oils by MAE were compared with those obtained using conventional extraction HD technique. MAE offered reduction in the extraction time with better oil yield (1.2 % w/v) than that obtained when compared to HD (0.7 % w/v). Using MAE and HD for the extraction of *E. sanctae-catharinae* essential oil showed difference in the composition and percentage composition of the oil obtained with valencene (16.01%) and butyl hydroxy toluene (25.58%) being the major contents of the extracted oil using HD and MAE, respectively. **Conclusion:** To the best of our knowledge, this is the first study for the essential oil from *E. sanctae-catharinae*. The tested essential oil samples did not show any significant antimicrobial activity at a concentration of 1 mg per disc.

Keywords: *Antimicrobial, Essential oils, Euphorbia sanctae-catharinae, Hydro-distillation, Microwave-assisted.*

INTRODUCTION

Euphorbiaceae family, one of the largest families, is very diverse in range, composed of a wide variety of plants ranging from large woody trees to simple weeds that grow prostrate to the ground. The family composed of over 315 genera and nearly 8,000 species[1]. *Euphorbia* species have been widely used in folk medicine for treatment of diarrhea, inflammation, and swellings and is known as a wart remover[2-4]. Some of these species are endemic or confined in Egypt to the Sinai Peninsula[5-6]. As no reports were found to be done for the oil contents of *E. sanctae-catharinae*, particularly this variety growing wild in the mountains of Sinai (Gebal Sait Katherine), it was motivating to the authors to undertake this study.

Microwave-assisted extraction (MAE) is a superior greener alternative to several thermal extraction techniques. Early reports, to extract essential oils using MAE, were recorded in the 80's[7]. Recently, it has been used by several authors to replace conventional

extraction techniques to extract essential oils from different medicinal and aromatic plants (MAPs)[8-10]. Recent studies have witnessed the development of microwaves applications in the separation and extraction of MAPs; such as microwave hydro-diffusion and gravity, solvent free microwave-assisted distillation and compressed air microwave-assisted distillation[11-13]. Recent trends in the use of microwave energy as alternative green extraction technology has largely focused on finding solutions to overcome conventional extraction methods drawbacks. The advantages of extraction using microwave energy, owing to its unique effective mechanism which is a noncontact heat source to produce and deliver heat to the extraction matrix, include more effective heating, faster heat energy transfer, reduced thermal degradation, increased extraction selectivity, reduced equipment size, more rapid start-up to the extraction process and increased yield production[14]. Therefore, the aim of this study was to evaluate the effect of MAE on the composition and the percentage composition of *E. sanctae-catharinae* essential oil and compare the obtained results with essential oil obtained by conventional extraction technique; hydro-distillation (HD).

MATERIALS AND METHODS

Plant Material

Aerial parts of *Euphorbia sanctae-catharinae* Fayed, were collected in June 2013, from North Sinai, Egypt and the plant was kindly authenticated by Dr. Mona Marzouk, Associate Professor of Taxonomy, National Research Centre, Cairo, Egypt. A voucher specimen has been deposited in the Herbarium of National Research Centre, Egypt (voucher ID 212). Collection was taken place under the permission of Saint Katherine protectorate for scientific purposes.

Microwave-assisted extraction

The extraction of the essential oil was carried out using focused microwave apparatus (CEM Corporation, Matthews, NC, USA), model (MARS 240/50, No. 907511, frequency 2450 MHz) operating at 2450 MHz with maximum power 1600 W. 100 g of sample (dried aerial parts of *E. sanctae-catharinae*) were placed in a 5000 mL round-bottomed flask that connected to Clevenger- type apparatus outside of microwave oven**.** The extraction was operated using 800 W power for 60 min. Temperature was adjusted at 100 °C. The essential oil was recovered and its volume was determined using micropipette. The obtained yield was calculated as percentage (volume of recovered oil per weigh of sample). Obtained oil was dried on anhydrous sodium sulfate to remove excess water and saved in a refrigerator till analysis.

Hydro-distillation extraction

For comparison, HD extraction of the essential oil was carried out using 100 gm of dried aerial parts of *E. sanctae-catharinae.* Extraction was carried out for 3 hours using a Clevenger-type apparatus. After HD extraction, same post extraction procedures for MAE, were applied on the recovered oil.

Essential oils chemical composition by Gas chromatography-mass spectrometry analysis

The Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 mL/min and a split ratio of 1:10 using the following temperature program: 60 °C for 1 min; rising at 4.0 °C/min to 240 °C and held for 1min. The injector and detector were held at 200 °C. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450.

Identification of essential oil constituents

The identification of the chemical constituents of the essential oil were deconvoluted using AMDIS free software (www.amdis.net) and identified by its retention indices (relative to *n*-alkanes C_8-C_{22}), mass spectrum matching to authentic standards (when available)., and Wiley Spectral Library Collection and NSIT library database).

Antimicrobial activity

Microorganisms

The microorganisms used in this study were obtained from the culture collection of Microbial and Natural Products Chemistry Department, National Research Centre (NRC), Giza, Egypt. Gram positive bacterium: *Staphylococcus aureus* ATCC and Gram negative bacterium: *Escherichia coli* ATCC 25922 were used for testing the antibacterial activity. While, the antifungal activity was tested against *Aspergillus niger* NRRL-599 and *Candida albicans*. The bacterial strains were incubated in nutrient agar medium at 37°C for 24 hr, while those of fungi were incubated on potato dextrose agar at 28°C for 72 hr.

Chemicals

The nutrient agar and potato dextrose agar media were purchased from Lab M limited (Lancashire BL9 6AS, UK) and Becton, Dickinson and company Sparks (MD 21152, USA), respectively. The positive controls, thiamphenicol and nystatin, were obtained from Sanofi-Aventis and Pharaonia Pharmaceutical companies, Egypt, respectively and used in a concentration of 50 µg/disc.

Antimicrobial activity

The antimicrobial activity was carried out using agar disc diffusion method[15]. The bacterial and fungal suspensions were swabbed and spread on nutrient agar and potato dextrose agar, respectively and adjusted to McFarland No. 0.5 standard turbidity. The sterilized paper discs (6 mm D) with the required doses of the methanolic solution of the essential oil (250, 500 and 1000 µg/disc) were placed on the surface of inoculated plates. The positive controls, thiamphenicol and nystatin (50 µg/disc), were used for determination of the sensitivity of bacteria and fungi, respectively.

RESULTS AND DISCUSSION

Extraction time and yields

From the obtained results, MAE is clearly faster than HD extraction. The extraction using MAE is 3 times faster than HD extraction. In addition, only 10 min using MAE was enough to reach the extraction temperature, which is equal to boiling temperature of water (100°C); however, 30 min was required by HD extraction to reach the boiling temperature of water. Moreover, an extraction time of 60 min using MAE offered better yield than obtained using HD extraction for 3 hrs (1.2 and 0.7 % (w/v); respectively), confirming significant saving of extraction time and energy consumed.

Composition of essential oil

The composition and the percentage composition of *E. sanctae-catharinae* essential oil extracted using HD and MAE, presented in Table 1 in order of their elution from the column. Figures (1a) and (1b) show the chromatogarms of essential oils of *E. sanctae-catharinae* isolated by HD and MAE; respectively.

Forty-nine components were identified in oil extracted using MAE, representing 95.4% of the oil obtained, while 35 components were identified in oil extracted using HD, representing 95.57% of the oil obtained.

The main composition and the percentage composition of *E. sanctae-catharinae* essential oil extracted using HD and MAE were identified as follow; α-pinene (4.18% and 0.02%), limonene (7.66% and 0%), alloocimene (2.26% and 0.02%)

Table 1. The composition and the percentage composition of *E. sanctae-catharinae* essential oil prepared by hydro-distillation and Microwave-assisted extraction

No	Class	Component	L.R.I*	RT	Relative content (%)	
					HD	MAE
1	MH	α-Pinene	909.3	4.78	4.18	0.02
2	MH	2-β-Pinene	951.8	5.97	0.21	0.01
3	MH	Sabinene	983.0	6.84	1.08	0.01
4	MH	Limonene	1007.1	7.58	7.66	0
5	MH	Alloocimene	1007.5	8.60	2.26	0.02
6	OM	Linalool oxide	1145.4	13.07	-	0.14
7	OM	trans-β-terpineol	1156.6	13.56	-	0.02
8	OM	α-Terpineol	1173.0	14.26	-	0.08
9	OM	cis-p-mentha-1(7),8-dien-2-ol	1199.7	15.43	0.76	0.02
10	OM	Chrysanthenyl acetate	1225.6	16.60	2.51	0.01
11	OM	Thymol (CAS)	1263.0	18.30	-	7.00
12	OM	Carvacrol	1272.3	18.72	-	1.03
13	OS	α-Muurolene	1325.0	21.08	0.74	0
14	SH	α-Copaene	1330.5	21.37	0.96	0.07
15	OS	cis-z-α-Bisabolene epoxide	1366.6	22.96	-	0.17
16	SH	Longifolen	1367.7	23.00	1.35	0
17	SH	β-Caryophyllene	1371.7	23.18	1.48	1.19
18	SH	β-copaene	1382.1	23.65	0.84	0
19	SH	Junipene	1402.5	24.55	0.61	0
20	SH	Humulene	1406.1	24.72	-	0.22
21	SH	β-Chamigrene	1407.5	24.77	1.80	0
22	SH	Aromadendrene	1409.3	24.85	0.90	0.43
23	OS	Campherenone	1414.4	25.07	-	0.06
24	SH	Valencene	1423.9	25.48	16.01	0.36
25	SH	Germacrene-D	1430.5	25.77	-	1.00
26	OS	(*E*)-β-Ionone	1434.0	25.92	0.51	0.54
27	OS	β-Ionol	1437.3	26.06	-	0.39
28	SH	β-Selinene	1439.3	26.14	0.80	0
29	OS	Cubedol	1445.4	26.41	-	0.18
30	SH	β-Cedrene (CAS	1448.2	26.53	0.60	0.18
31	OS	Butyl hydroxyl toluene	1452.9	26.73	-	25.58
32	OS	Diepicedrene-1-oxide	1456.0	26.86	1.30	0.83
33	SH	γ-Cadinene	1461.7	27.11	1.20	0.19
34	SH	δ-Cadinene	1465.6	27.28	1.48	1.56
35	OS	α-Cedrol	1474.1	27.66	0.85	0.24

Table 1. Continue

No	Class	Component	L.R.I*	RT	Relative content (%)	
					HD	MAE
36	OS	Widdrol hydroxy ether	1478.9	27.85	-	0.26
37	OS	Isoaromadendrene epoxide	1496.3	28.60	0.62	0.27
38	OS	trans-*Z*-*α*-Bisabolene epoxide	1503.5	28.91	1.34	0.60
39	OS	1,5-epoxysalvial-4(14)-ene	1513.4	29.32	1.75	0.49
40	OS	(+) spathulenol	1522.3	29.69	15.41	10.32
41	OS	(-)-Caryophyllene oxide	1525.8	29.84	10.50	3.69
42	OS	salvial-4(14)-en-1-one	1537.6	30.32	4.11	0.98
43	OS	Globulol	1547.2	30.71	-	0.43
44	OS	Epiglobulol	1553.0	30.95	0.52	0.65
45	OS	Neoclovenoxid-alkohol	1560.4	31.26	-	1.23
46	OS	Ledene oxide-(II)	1561.9	31.32	2.80	0
47	OS	Cubenol	1569.2	31.62	0.43	0.33
48	OS	tau.-Cadinol	1583.4	32.21	-	2.32
49	OS	*α*-Cadinol	1585.4	32.29	-	1.23
50	OS	Calarene epoxide	1590.4	32.50	0.47	1.24
51	OS	*β*-Eudesmol	1595.6	32.71	4.36	13.67
52	OS	Aromadendrene oxide-(2)	1599.7	32.87	-	0.75
53	OS	Cedren-13-ol, 8-	1615.0	33.48	-	0.31
54	OS	6-epi-shyobunol	1631.6	34.14	3.18	11.83
55	OS	Aromadendrene oxide-(1)	1643.3	34.60	-	0.53
56	OS	Hexahydrofarnesyl acetone	1766.3	39.32	-	1.67
57	OS	Isobutyl phthalate	1787.2	40.1	-	1.05
Total identified					95.57	95.4
Monoterpene Hydrocarbons (MH))					15.39	0.06
Oxygenated Monoterpenes (OM)					3.26	8.30
Sesquiterpene Hyrocarbons (SH)					28.03	5.20
Oxygenated Sesquiterpenes (OS)					48.89	81.67

**LRI: Linear retention Index*

The main composition and the percentage composition of *E. sanctae-catharinae* essential oil extracted using HD and MAE were identified as follow; α-pinene (4.18% and 0.02%), limonene (7.66% and 0%), alloocimene (2.26% and 0.02 %), chrysanthenyl acetate (2.51% and 0.01%), thymol (0 and 7%) , valencene (16.013% and 0.36%), butyl hydroxy toluene (0% and 25.58%), (+) spathulenol (15.41% and 10.32%), (-) caryophyllene oxide (10.50% and 3.69%), salvial-4 (14)-en-1-one (4.11% and 0.98%), ledene oxide-2 (2.80% and 0%), *β*-eudesmol (4.36% and 13.67%) and 6-epi-shyobunol (3.18% and 11.83%); respectively.

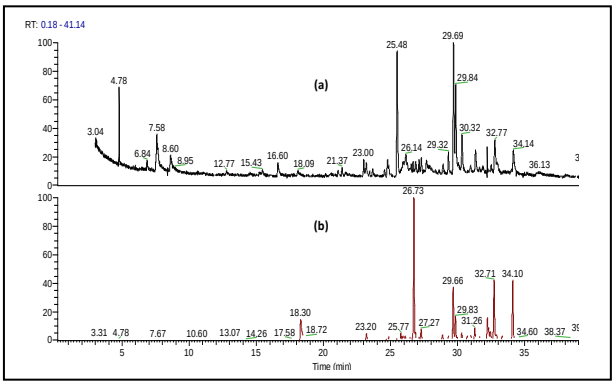

Figure 1: The GC/MS chromatogram of the essential oils obtained from dried aerial part of *E. sanctae-catharinae;* (a): Hydro-distillation extraction and (b) Microwave-assisted extraction

Comparison of both extraction methods (HD and MAE) showed that [ledene oxide-2 (2.8%), α-muurolene (0.74%), longifolen (1.35%), *β*-copaene (0.84%), junipene (0.61%), *β*-chamigrene (1.8%) and *β*-selinene (0.8%)] were missing in essential oil extracted using MAE. However, some new compounds [linalool oxide (0.14%), trans-*β*-terpineol (0.02%), *α*-terpineol (0.08%), thymol (7%) and carvacrol (1.03%), Humulene (0.22%), Campherenone (0.06%), Germacrene-D (1%) and butyl hydroxyl toluene (25.58%)] obtained by MAE were missing in essential oil extracted by HD.

Antimicrobial activity

Results of evaluation of antimicrobial activity as presented in Table 2 of the tested samples of MAE and HD essential oils in different doses; 250, 500 and 1000 µg/disc have shown negative antibacterial activity when compared to thiamphenicol standard (50 µg/disc) against

Table 2. Evaluation of antimicrobial activity of MAE and HD *E. sanctae-catharinae* essential oils using agar disc diffusion method

Sample	Minimum inhibitory concentration MIC (µg/disc)			
	St. aureus	*E. coli*	*C. albicans*	*A. niger*
MAE	0	0	0	0
HD	0	0	0	0
Thiamphenicol	5	2.5	0	0
Nystatin	0	0	2.5	>5

MAE=essential oils obtained by microwave-assisted extraction (used doses; 250, 500 & 1000 µg/disc). HD=essential oils obtained by hydro-distillation (used doses; 250, 500 & 1000 µg/disc).

both Gram positive bacterium (*Staphylococcus aureus)* and Gram negative bacterium (*Escherichia coli)* and negative antifungal activity when compared to nystatin (50 µg/disc) standard against both *Aspergillus niger* and *Candida albicans*.

CONCLUSION

This is the first report of essential oil composition of *E. sanctae-catharinae*, showing microwave-assisted extraction (MAE) method to be an efficient alternative method for the extraction of *E. sanctae-catharinae* essential oil than conventional hydro-distillation extraction (HD). However, MAE and HD extraction methods of *E. sanctae-catharinae* essential oil showed differences in both composition and percentage constituents of the obtained oils. Valencene (16.01%) and butyl hydroxy toluene (25.58%) are dominant compounds of the extracted oils using HD and MAE; respectively. MAE offered several advantages over conventional hydro-distillation (HD); shorter extraction time (60 min *vs* 3hrs) and better extraction yield (1.2 *vs* 0.7 w/v). Essential oil of *E. sanctae-catharinae* has neither antibacterial nor antifungal activity.

Conflict of Interest

The authors declare no conflict of interest.

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